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(21) International Application Number: PCT/AU97/00214 (22) International Filing Date: 3 April 1997 (03.04.97) (30) Priority Data: PN 9123 3 April 1996 (03.04.96) AU (71) Applicants (for all designated States except US): ST. VINCENT INSTITUTE OF MEDICAL RESEARCH [AU/AU]; 9 Princes Street, Fitzroy, VIC 3065 (AU). ST. VINCENT'S HOSPITAL [AU/AU]; Victoria Parade, Fitzroy, VIC 3065 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): POWER, David, Anthony [AU/AU]; 30 Irymple Avenue, Kew East, VIC 3102 (AU). KEMP, Bruce, Ernest [AU/AU]; 20 Kellett Grove, Kew, VIC 3101 (AU). (74) Agent: F.B. RICE & CO.; 28A Montague Street, Balmain, NSW 2041 (AU).		(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: IMMUNOLOGICAL REJECTION OF TRANSFECTED TUMOUR CELLS (57) Abstract The present invention provides a method of inducing an immune response against human tumour cells which can be used in the treatment of tumours in humans. The method involves transfecting the tumour cells with a polynucleotide encoding a glycosyltransferase. The glycosyltransferase may be selected from α -1,3 galactosyltransferase, α -N-acetyl-D-galactosaminyltransferase and α -D-galactosyltransferase.		

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"IMMUNOLOGICAL REJECTION OF TRANSFECTED TUMOUR CELLS"

The present invention relates to a method of inducing an immune response against human tumour cells. The present invention also relates to methods of treatment of tumours in humans.

5 Various non-surgical methods of treating tumours in humans have been developed and trialed in recent years. Such methods include, for example, radiotherapy and/or chemotherapy; photoradiation therapy; thermotherapy, neutron therapy; the use of monoclonal antibodies for selectively identifying and destroying neoplastic cells; and the
10 administration of recombinant hormones and cytokines such as α -interferon, β -interferon and interleukin-2.

More recently, the possibility of persuading the body's immune system to destroy tumour cells has been recognised. For this to happen, the immune system must be able to pick out tumour cells and treat them as
15 foreign. To this end, preliminary results indicate that direct injection of cDNA encoding HLA-B7, a transplantation antigen, into solid cutaneous tumours leads to rejection of transfected cells and formation of an immune response against non-transfected cells at other sites.

It is well documented that a major problem with the transplantation
20 of organs from one discordant species to another (xenograft transplantation) is hyperacute rejection. This is mediated by preformed antibodies to specific donor organ antigens causing activation of the complement system and cell lysis. It is thought that a major contributor to the immune response against xenografts in humans is the Gal (1.3) Gal epitope present on bacteria and
25 cells of all vertebrates below old world monkeys. This epitope is formed by a specific galactosyltransferase (α (1.3) galactosyltransferase). In humans, the α (1.3) galactosyltransferase gene is a pseudogene and the enzyme is not expressed. A natural anti-Gal antibody is present in the serum of all humans. This anti-Gal antibody constitutes approximately 1% of circulating IgG in
30 humans and interacts specifically with the Gal (1.3) Gal epitope (Galili *et al* (1993); Hamedeh *et al* (1992)).

The porcine α (1.3) galactosyltransferase gene has been cloned and sequenced and is described in Strahan *et al* (1995) and PCT/AU94/00126, the entire disclosures of which are incorporated herein by reference.

35 Another immune response which leads to cell destruction occurs in the transfusion of blood into a recipient who has antibodies against the donor

blood components. An individual of a particular ABO blood group can recognise red cells carrying different blood group antigens and produce antibodies to them. Antibodies may be produced naturally, without immunisation with the foreign red cells. Table 1 indicates the genotypes and antigens of the ABO system. Most people naturally make antibodies to the antigens they lack.

blood group (phenotype)	genotypes	antigens	antibodies to ABO in serum
A	AA,AO	A	anti-B
B	BB,BO	B	anti-A
AB	AB	A and B	none
O	OO	H	anti-A and anti-B

Table 1

The antigenic properties of these blood-group substances are determined by terminal sugars on an oligosaccharide chain built up of D-galactose, D-glucose, N-acetyl-D-glucosamine, N-acetyl-galactosamine and L-fucose. Briefly, the enzyme produced by the H transferase gene attaches a fucose residue (Fuc) to the terminal galactose (Gal) of the precursor oligosaccharide. Individuals possessing the A transferase gene (encoding α -N-acetyl-D-galactosaminyltransferase) attach N-acetyl galactosamine (NAGA) to this galactose residue while those with the B transferase gene (encoding α -D-galactosyltransferase) attach another galactose producing A and B antigens respectively.

The present inventors have found that human tumour cells stably transfected with a polynucleotide encoding porcine α (1,3) galactosyltransferase are more susceptible to lysis by human serum than non-transfected tumour cells. It is believed that human tumour cells stably transfected with a polynucleotide encoding an appropriate glycosyltransferase involved in the formation of the A and/or B blood group antigens will also be more susceptible to lysis by human serum.

Accordingly, in a first aspect the present invention provides a method of inducing an immune response against a human tumour cell which method includes introducing into the tumour cell a polynucleotide encoding a

glycosyltransferase such that the glycosyltransferase sequence is expressed in the tumour cell.

5 In a second aspect the present invention provides a method of treatment of tumours in humans which method includes introducing into the tumour cells a polynucleotide encoding a glycosyltransferase such that the glycosyltransferase sequence is expressed in the tumour cells.

In a preferred embodiment the glycosyltransferase is α -1,3 galactosyltransferase. Preferably, the α -1,3 galactosyltransferase is a porcine α -1,3 galactosyltransferase.

10 In another preferred embodiment the glycosyltransferase is selected from α -N-acetyl-D-galactosaminyltransferase and α -D-galactosyltransferase.

Membrane regulatory proteins such as CD55 (DAF - decay-accelerating factor), CD46 (MCP - membrane cofactor protein) and CD59 (membrane inhibitor of reactive lysis) are found on many human cell types and are thought to act as natural inhibitors of complement activation and cell lysis (Shin *et al* (1986); Hourcade *et al.* (1989)).

20 Accordingly, in another preferred embodiment the method further includes treating the tumour cells with a suppressor of a natural inhibitor of complement activation. Suppressors of these inhibitors include antibodies which bind to the inhibitory proteins. The suppressor may, for example, be a monoclonal antibody which binds to the inhibitory protein CD55, CD46 or CD59.

The polynucleotide encoding the glycosyltransferase may be introduced into the tumour cell by any suitable method of gene delivery. Suitable methods of gene delivery may, for example, involve the use of viral vectors. Viruses can be modified to carry a desired gene and become vectors for gene delivery. International publication nos. WO 95/07994 and WO 94/29469 disclose vectors which have been adapted for delivery and expression of polynucleotides which encode immunogenic or therapeutic peptides. These vectors are particularly suitable for gene delivery to tumour cells.

35 As an alternative to using viruses, it is possible to transfer genes into human cells directly. For example, a desired gene may be inserted into a bacterial plasmid along with promoter, enhancer and other sequences that enable the gene to be expressed in human cells. The plasmid DNA can be

incorporated into lipid vesicles (Liposomes including cationic lipids such as lipofectin) which then transfer the DNA into the target cell.

DNA can also be complexed with proteins that target the DNA to specific tissues in the same way as certain proteins are taken up
5 (endocytosed) by specific cells. International publication No. WO 94/23751 describes a nucleic acid transfer peptide for efficient delivery of nucleic acid to cells which is particularly suitable for use in gene therapy of tumours.

Another gene delivery technique involves "shooting" naked DNA on small gold beads into a cell using a "gun".

10 It will be understood that the introduction of a polynucleotide encoding $\alpha(1,3)$ galactosyltransferase into a tumour cell according to the first aspect of the invention will result in the formation of the Gal (1,3) Gal epitope on the surface of the transfected cell. Use of the Gal (1,3) Gal epitope provides advantages over the use of other epitopes (such as HLA-B7) in
15 regard to the induction of an immune response in humans. The first advantage resides in the fact that humans produce a natural antibody, anti-Gal, which interacts specifically with α -galactosyl epitopes and which constitutes up to 1% of circulating immunoglobulins in humans. Secondly, the Gal (1,3) Gal epitope is produced in abundance ($>10^6$ per cell) on cells of
20 non-primate animals which naturally express the $\alpha(1,3)$ galactosyltransferase enzyme (Galili *et al.* (1988); Thall *et al.* (1991). Accordingly, human tumour cells expressing the Gal (1,3) Gal epitope are likely to be quickly recognised as foreign and strongly attacked by the immune system in a manner analogous to rejection of xenograft transplants.

25 The mechanism by which the Gal (1,3) Gal epitope causes cell death is not fully understood. It is possible, however, that the presence of the epitope induces antibody dependent cell-mediated cytotoxicity (ADCC). This mechanism involves binding of cells with cytotoxic potential to target cells coated with an antibody. ADCC generally causes rapid cell death and
30 has been implicated in reported instances of cell destruction by anti-gal antibodies. For example, Galili (1993) demonstrated the destructive effect of anti-Gal on non primate cells in an *in vitro* ADCC assay. Incubation of affinity-purified human anti-Gal antibodies with porcine cells (endothelial, smooth muscle, fibroblasts) and T-cell depleted peripheral blood
35 mononuclear cells (PBMC) caused cell lysis at an effector:target ratio of 5:1 or greater. Significant lysis was not found with PBMC alone.

It will also be understood that the introduction of a polynucleotide encoding α -N-acetyl-D-galactosaminyltransferase and/or α -D-galactosyltransferase will result in the formation of the A and/or B antigens on the surface of the transfected cell. Tumour cells expressing the A and/or B antigens are likely to be quickly recognised and attacked in humans who naturally produce the anti-A or anti-B antibodies. Preferably, the tumour cells are engineered to produce the antigen which is reactive with the naturally occurring antibodies in the individual. For example, a tumour in an individual of blood group A is preferably transfected with a polynucleotide encoding α -D-galactosyltransferase which gives rise to the B antigen. It will be appreciated that individuals of the blood group phenotype AB do not naturally produce anti-A or anti-B antibodies. These individuals are therefore not ideal subjects in relation to this aspect of the invention. However, individuals of the AB phenotype are relatively rare, occurring at a frequency of only around 4%.

Although the anti-Gal, anti-A and/or anti-B antibodies occur naturally in most individuals, it would be possible to boost levels of any one of these antibodies by immunisation with the corresponding antigen. Higher levels of the antibodies would presumably lead to a quicker and more effective attack on tumour cells transfected with either the α -1.3 galactosyltransferase, α -N-acetyl-D-galactosaminyltransferase and/or α -D-galactosyltransferase genes.

In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following examples.

Example 1

Cell culture

A431 is an epidermoid carcinoma cell line. It was grown in 10% fetal calf serum in D-MEM. Cells were passaged by trypsinisation with 0.1% trypsin/EDTA.

Transfection of A431 with porcine gal transferase

A 1.2kb cDNA fragment containing the complete coding region for porcine gal transferase was the generous gift of Dr. Peter Cowan, St. Vincent's Hospital. It was obtained already cloned into the multiple cloning site in the

expression vector pcDNA3 (Invitrogen). A431 cells were transfected using the DEAE-dextran method and transfected cells selected using media containing the antibiotic G418. After 2 weeks in culture, cells were subcloned and colonies selected according to the results of FACS analysis using FITC-labelled IB4 lectin (Sigma), which detects the gal ($\alpha 1,3$) gal linkage. A line (A431.gal) was derived after two rounds of cloning. 75% of cells from the line were positive in flow cytometric assay using FITC-conjugated IB4 lectin, when compared with non-transfected cells.

10 Activation of complement by incubation of transfected cells with human serum

To detect activation of complement on the surface of A431.gal cells by flow cytometry, A431.gal or control non-transfected A431 cells were incubated for 30 mins at 23°C with 0-50% (v/v) normal human serum (NHS; blood group AB) and then washed x1 in wash buffer. They were then analysed for (1) complement binding to the cell surface using a flow cytometric assay for C3c and C9, and (2) cell viability using (i) uptake of the vital dye calcein AM (Molecular Probes) by flow cytometry, and (ii) cell proliferation by incorporation of ^3H -thymidine.

20

(1) Detection of complement binding to cell surface using FACS

Gal transfected A431.gal cells or non-transfected control A431 cells were suspended in 50 μl staining buffer comprising wash buffer (KDS-BSS/10% HI-FCS/0.01% Nan3) with 10% normal mouse serum at approximately 1×10^5 cells per ml in V-bottomed tubes (Biolab Scientific). A predetermined dilution of FITC-conjugated mouse monoclonal antibody to C3c, C9 was used to detect binding of early or late complement components, respectively. Stained cells were analysed in a FACScan flow cytometer (Becton Dickinson). Results were analysed using either the Lysis II or Cell-Quest programs (Becton Dickinson).

30

(2) Assay for cell viability using calcein AM (CAM)

CAM is a nonfluorescent cell permeable substrate that is converted in the presence of intracellular esterase to intensely fluorescent calcein. This assay was used to detect the number of live cells. Calcein AM is a red fluorochrome which is a vital dye, so that only viable cells will take it up.

35

The assay was performed using the manufacturer's instructions. Briefly, $1\mu\text{M}$ calcein AM was added to 3×10^5 cells in a volume of $100\mu\text{l}$ for 20 mins at 23°C , washed and analysed on the flow cytometer.

- 5 (3) Assay for cell viability using incorporation of ^3H -thymidine
A431.gal or A431 cells were grown in 24-well plates and then treated with 0-50% human AB serum for 30 mins at 23°C , the medium was changed and the cells then pulsed with $1\mu\text{Ci}$ of ^3H -thymidine for 4 hours. Cells were harvested using NaOH and $50\mu\text{l}$ added to liquid scintillant and counted in a
10 beta counter.

Results

- 15 1. Presence of the complement regulatory molecules CD55 (DAF), CD46 (MCP) and CD59 on the surface of A431 cells.
An initial step was assay of A431 cells for the presence of the membrane regulatory proteins CD55 (DAF), CD46 (MCP), and CD59 by flow cytometry. These proteins are found on many human cell types and are thought to act as natural inhibitors of complement activation and cell lysis. By FACS analysis,
20 A431 cells were found to express high levels of all 3 of these proteins. This was important as future studies blocking the function of these inhibitory molecules could lead to increased susceptibility of the cells to lysis.

- 25 2. Binding of C3c and C9 in A431 and A431.gal following incubation with human serum.
Following incubation with NHS, A431 cells did not possess detectable C3c or C9 on the cell surface. Transfected A431.gal cells showed some binding of C3c with concentrations of NHS $< 10\%$. However, even with very high serum concentration (up to 50%), C9 was not detected.

- 30 3. Detection of cell death in A431.gal cells treated with serum

Two methods were used;

(1) Thymidine Incorporation (cell proliferation)

- 35 Non-transfected and transfected cells were placed in wells with NHS. Thymidine was added to the wells and after 4 hours cells were harvested and thymidine uptake was assessed. There was no significant difference in the

rate of thymidine uptake between the transfected and non-transfected cells indicating negligible lysis of the transfected A431.gal cells.

(2) CAM (Calcein-AM) Assay

5 A431 and A431.gal cells were incubated with varying concentration of NHS for 4 hours and then labelled with CAM. No detectable cell death occurred and there was no difference between transfected and non-transfected cells.

(4) Detection of cell death in A431.gal cells treated with serum and a blocking antibody of CD55

10 The next series of experiments was aimed at enhancing the possibility of cell lysis of transfected cells by adding a monoclonal antibody (mAb) blocker of the inhibitory protein CD55. Recent studies have indicated that the homologous complement restriction factors CD46, CD55, and CD59, which are integral membrane proteins, play a significant role in suppressing
15 complement-mediated cell lysis by homologous serum. The first mAb used was directed against CD55. With the addition of increasing concentrations of anti-CD55 mAb there was a significant increase in cell binding of C9. There was also an increase in the number of dead A431.gal cells when they were co-incubated with NHS and anti-CD55, using the CAM assay.

20

Example 2

Experiments were performed to determine if (1) human skin cancer cells (A431) and (2) breast cancer cells (MDA-MB-435(MDA cells)) stably
25 transfected with cDNA encoding pig Gal (1,3) Galactosyl transferase and having been found to express Gal (1,3) Gal epitope were more susceptible to complement mediated lysis than non transfected tumour cells, given 1% of preexisting human IgG antibodies are directed against the Gal (1,3) Gal epitope.

30

Transfection

(a) MDA cell transfection was conducted using the porcine gal transferase described in Example 1. Fifteen positive clones were obtained.
35 (B) Previously transfected and non-transfected A431-gal cells were obtained from cryostorage.

Gal Expression

5 FACs were performed (as described in Example 1) on the MDA-gal clones, non-transfected MDA cells, A431-gal cells and non-transfected A431 cells to determine the levels of gal expression.

Method:

10 Gal clones were treated with FITC-labeled IB4 lectin, binding to the gal epitope. Fluorescence was measured using FACs.

Results:

15 Of the 15 MDA-gal clones tested, 10 yielded positive results and 5 negative. Of the gal-positive clones, MDA-gal clones, I, J and E were chosen to be grown and used in subsequent experiments. J and I exhibited the highest mean level and second highest mean level of gal expression respectively, upon analysis of results. E was chosen based on its most uniform level of gal expression across the population (well defined histogram peak). Of the negative results, MDA-gal clone L was chosen.

20 A431-gal cells showed a more depressed level of gal expression compared to MDA-gal I, J, or E with greater spread or variation within the population.

25 Both the non-transfected MDA and A431 cells yielded negative results.

CRF Assay

30 FACs was performed on the MDA and A431 cells to determine the presence and levels of membrane regulatory proteins CD55 (DAF), CD46 (MCP) and CD59, thought to act as natural inhibitors of complement activation and cell lysis. Previous attempts at complement mediated cell lysis of A431-gal cells using homologous normal human serum (NHS) showed negative results, whereas blocking these inhibitory proteins with monoclonal antibodies showed increased binding of terminal components in complement cascade and cell lysis.

35

Method:

Cells were treated with FITC-labeled antibodies to CD55, CD59 and CD46 for 30 minutes. FACs performed.

5 Results:

Both the MDA and A431 cell lines showed high levels of all three. CD55 and CD46 expression was the highest and lowest respectively. Levels of expression in both the MDA and A431 cells were very similar.

10 Cytotoxicity (LDH) Assay

Cytotoxicity assays were done on the MDA and A431 cell lines, using the lactic dehydrogenase (LDH) release method. Cells were transfected with either galactosyltransferase or control construct. Two lines were used for
15 MDA (one high and one low level expressor), and one line for A431.

Method:

A normalised number of non-transfected and transfected cells were placed in wells with 0% and 20% NHS. Positive controls were obtained by freezing (-
20 70°C) and thawing cell twice. The LDH kit was used to detect levels of LDH (an intracellular enzyme) released into the supernatant following cell lysis to determine the degree of cell lysis. Absorbance measurements were done with the microplate reader. % Cytotoxicity values were then calculated using the formula as per the protocol.

25

Results:

Using the same supernatant, LDH assays were performed. There was greater lysis in the cells transfected with galactosyltransferase (12-18% and 36-47%
30 for the two transfected lines), compared with the control (3-8%).

30

For the A431-gal and its vector control the calculated results yielded negative %s. in both assays, due to a high spontaneous release of LDH from the cells. However, consistently higher results were found in the cells transfected with galactosyltransferase than in the controls, so that these cells had probably
35 been killed by 20% human serum, as well.

Summary

Using A431 and MDA cells transfected with porcine gal transferase, preliminary results suggest that the cancer cells were more susceptible to cell lysis by human serum. There was increased binding of C3c to A431.gal cells transfected with galactosyl transferase, although binding of C9 was not increased, suggesting that homologous complement regulatory molecules present on the surface of A431.gal may have inhibited formation of the membrane attack complex. However, using a combination of normal human serum and a monoclonal antibody against CD55, there was increased binding of C9 to the transfected cells. Preliminary studies suggest that this combination may result in tumour cell death *in vitro* and *in vivo*.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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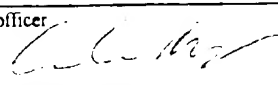
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CLAIMS:

1. A method of inducing an immune response against a human tumour cell which method includes introducing into the tumour cell a polynucleotide encoding a glycosyltransferase such that the glycosyltransferase is expressed in the tumour cell.
5
2. A method of treatment of tumour cells in a human which method includes introducing into the tumour cells a polynucleotide encoding a glycosyltransferase such that the glycosyltransferase sequence is expressed in the tumour cells.
10
3. A method according to claim 1 or claim 2 in which the glycosyltransferase is α -1,3 galactosyltransferase.
- 15 4. A method according to claim 3 in which the α -1,3 galactosyltransferase is a porcine α -1,3 galactosyltransferase.
5. A method according to claim 1 or claim 2 in which the glycosyltransferase is selected from α -N-acetyl-D-galactosaminyltransferase and α -D-galactosyltransferase.
20
6. A method according to any one of claims 1 to 5 which further includes treating the tumour cells with a suppressor of a natural inhibitor of complement activation.
25
7. A method according to claim 6 in which the suppressor is an antibody directed against CD55, CD46 or CD59.
8. A method according to any one of claims 1 to 7 in which the tumour cells are selected from human skin cancer cells and human breast cancer cells.
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INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/AU 97/00214

A. CLASSIFICATION OF SUBJECT MATTER				
Int Cl ⁶ C12N 15/54, A61K 45/05; A61K 48/00				
According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED				
Minimum documentation searched (classification system followed by classification symbols) SEE ATTACHED EXTRA PAGE				
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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) SEE ATTACHED EXTRA PAGE				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
X	WO 91/03484 (The Biomembrane Institute) 21 March 1991	1-8		
X	Glycobiology, 1995, volume 5, number 8, pages 775-782, U. Galili & F. Anaraki: "α-Galactosyl (Gal α1-3Galβ1 - 4GlcNAc-R) epitopes on human cells: synthesis of the epitope on human red cells by recombinant primate α1,3galactosyltransferase expressed in E-coli"	1-8		
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Date of the actual completion of the international search 3 July 1997		Date of mailing of the international search report 07 JUL 1997		
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (06) 285 3929		Authorized officer  CARMELA MONGER Telephone No. (06) 283 2434		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/AU 97/00214

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
P. X	Cancer Research, July 1 1996, volume 56, pages 3069-3074. D.C.LaTemple (et al). "Synthesis of α -Galactosyl epitopes by recombinant α 1,3-Galactosyltransferase for opsonization of human tumor cell vaccines by anti-galactose."	1-8
A	International Journal of Biochemistry, 1995, volume 27, number 3, pages 329-336. S.K.Chatterjee (et al), "Analysis of the sequences of human β -1,4-galactosyltransferase cDNA clones."	
A	Biochemical & biophysical research communications, May 15 1991, volume 176, number 3. L.Mangle-Gaw (et al) "Genomic structure & expression of human β -1,4-galactosyltransferase."	

Continuation of "B. FIELDS SEARCHED"

FIELDS SEARCHED

WPAT search terms	[Glycosyltransferase# or galactosyltransferase# or galactosaminyltransferase# or fucosyltransferase#] and [cancer; or neoplasm# or carcinoma# or tumour]
Medline search terms	[galactosaminyltransferase# or galactosyltransferase# or glycosyltransferase#] and [cancer/CT or neoplasm/CT]
Chem Abs search terms	[galactosaminyltransferase# or galactosyltransferase# or glycosyltransferase#] linked to "Biological studies/ROLE" and [neoplasm/IT or carcinoma/IT] and [1990 - 1997]

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 97/00214

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information

Patent Document Cited in Search Report	Patent Family Member	
WO 91/03484	AU 63372/90	EP 489822
END OF ANNEX		